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A new ectoparasitic distichodontid of the genus *Eugnathichthys* (Characiformes: Citharinoidei) from the Congo basin of central Africa, with a molecular phylogeny for the genus

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Abstract

A new species of ectoparasitic distichodontid, *Eugnathichthys virgatus*, is described from localities in the central and western Congo basin. The new species is a fin-eater even at small sizes and, in common with congeners, is capable of biting off sections of heavily ossified fin-rays of large prey species. Prior to the present study, two species were included in this distinctive distichodontid genus: the type species, *Eugnathichthys eetveldii*, and a second species, *E. macroterolepis,* both of which are widely distributed throughout much of the Congo basin. Morphologically, *E. virgatus* is readily distinguished from its two congeners based on a combination of meristic and morphometric attributes. The new species possesses a unique pigmentation pattern, a reduced number of pectoral-fin rays, and a markedly reduced dentition on the fifth ceratobranchial elements of the pharynx, all of which are derived features considered diagnostic for the new species. With molecular data the species is further diagnosed by four apomorphic, non-synonomous nucleotide transitions in two sampled genes (NADH dehydrogenase subunit 2 and glycosyltransferase). Phylogenetic analysis of those mtDNA and ncDNA markers supports a sister-group relationship between *E. virgatus* and *E. eetveldii* rather than with *E. macroterolepis*, the species with which it bears closest phenetic similarity.

Key words: *Eugnathichthys virgatus*, new species, ectoparasite, generic intrarelationships

Resumé

Une nouvelle espèce de distichodontid ectoparasite, *Eugnathichthys virgatus*, est décrite à partir des localités du centre et de l'ouest du bassin du Congo. La nouvelle espèce se nourrit de nageoire, et cela même à petites tailles. Elle a en commun avec les autres espèces congénères la capacité de manger, par morsure, de morceaux de nageoire fortement ossifiés d'espèces proies de grande taille. Avant la présente étude, deux espèces avaient été incluses dans ce genre distinctif de distichodontid: il s'agit des espèces type, *Eugnathichthys eetveldii*, et *E. macroterolepis* qui, tous deux, sont largement distribuées dans une grande partie du bassin du Congo. Morphologiquement, *E. virgatus* se distingue facilement de ses deux congénères sur base d'une combinaison de caractères méristiques et morphométriques. Cette espèce possède un motif de pigmentation unique, un nombre réduit de rayons des nageoires pectorales et une dentition nettement réduite sur les éléments du cinquième cératobranchial du pharynx, qui sont tous des traits dérivés, considérés comme diagnostic pour la nouvelle espèce. De surcroît, l'espèce est encore diagnostiquée par la présence de quatre nucléotides apomorphes de transition, non-synonymes, dans les deux gènes testés (NADH déshydrogénase sous-unité 2 et glycosyltransférase). L'analyse phylogénétique de ces marqueurs mtADN et ncADN soutient que *E. virgatus* et *E. eetveldii* sont issus d'un même groupe et ont un lien de parenté étroit plutôt qu'avec l'espèce *E. macroterolepis* qui porte la plus proche similitude phénotypique.

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Introduction

Prior to the present study the distinctive distichodontid genus *Eugnathichthys* contained the type species, *E. eetveldii* (Figs. 1A, B), and a sole congener, *E. macroterolepis* (Figs. 1B, C). Both species, originally described by Boulenger in the late 1890's from few individuals, are now known to be widely distributed throughout much of the Congo basin and seemingly occur in sympatry over most of that range (Fig. 2). *Eugnathichthys eetveldii* and *E. macroterolepis* are readily distinguished from one another based on non-overlapping scale and fin-ray counts (Boulenger, 1909), as well as by distinctive pigmentation patterning and caudal fin coloration (Figs. 1B, C).

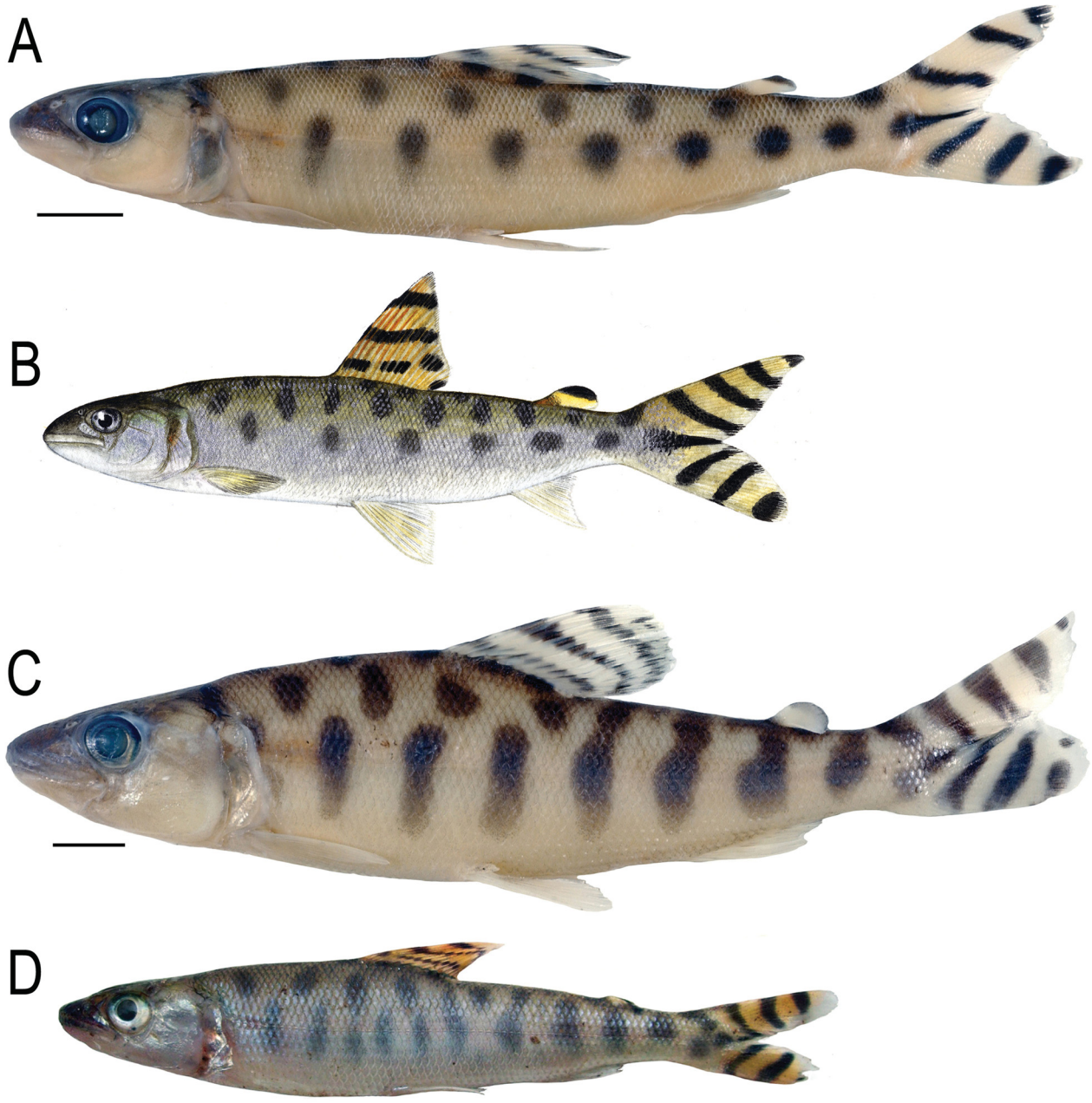

FIGURE 1. *Eugnathichthys*: (A) *E. eetveldii* (AMNH 243549, Lulua River), (B) *E. eetveldii*, James Chapin[1] watercolor of live specimen, Avakubi, Ituri River (specimen not retained), (C) *E. macroterolepis* (AMNH 254699, Nsele River), (D) *E. macroterolepis*, immediately post-mortem (AMNH 253624, Kwilu River). Reproduction of previously unpublished Chapin watercolor courtesy of the American Museum of Natural History, Library Archive.

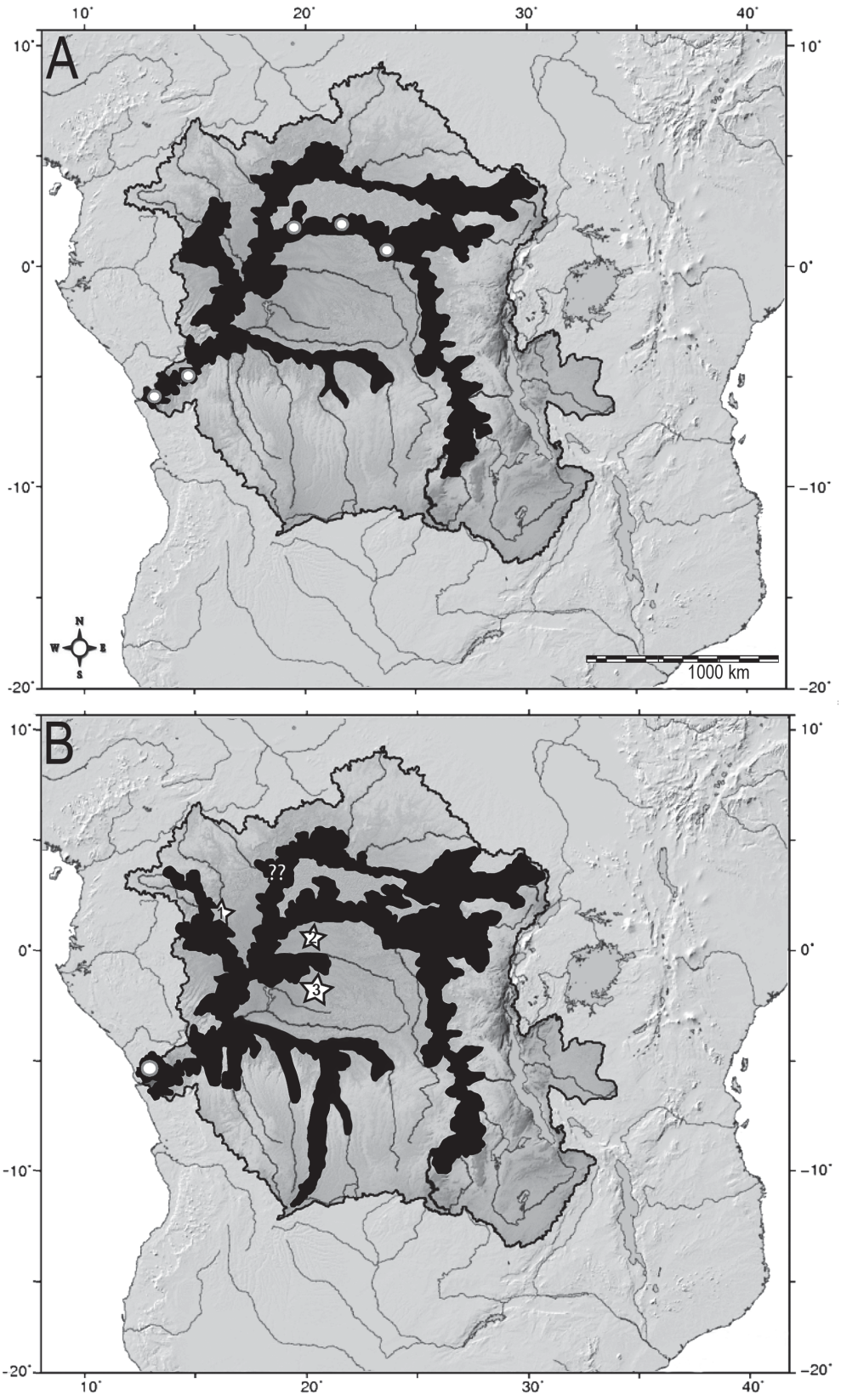

FIGURE 2. Distributional Ranges (modified after Brooks *et al.*, 2011), with Congo basin delimited in dark grey: (A) *Eugnathichthys eetveldii*, white circles indicate syntypical localities (B) *E. macroterolepis,* white circle indicates type locality, and stars indicate areas of occurrence of *E. virgatus* (1= Lengoué River, 2= Lomako River, 3= Luilaka and Yenge Rivers in Salonga National Park). "??" denotes possible occurrence in Mossapoula River (Ubangi basin), Central African Republic (see text).

Both *Eugnathichthys* species are reportedly ectoparasitic, feeding exclusively on fish fins as adults, although smaller individuals of *E. eetveldii* have been reported to eat aquatic insect larvae (Roberts, 1990). Vari (1979) provides a series of derived morphological features attesting to the monophyly of the genus, most notable of which are the presence of characteristically remodeled massive jaws and associated musculature, which facilitate a powerful bite employed during fin-feeding. Whereas nine other distichodontid species are known at least to be facultative, fin-eaters (Roberts, 1990), the massive jaws and robust shearing dentition of *Eugnathichthys* apparently allow these fishes to subsist exclusively on more robust, highly ossified fins than other fin-eating distichodontids, many of which are found to occur in sympatry with them.

In the course of a multi-locus molecular study of distichodontid intergeneric relationships four individuals identified as *E. macroterolepis* were included as terminals in that analysis (Arroyave *et al*., in press). An unanticipated degree of molecular divergence was observed between two individuals from the southern part of the species' range (Nsele and Kasai Rivers) and two individual from more northerly locations (Lengoué and Lomako Rivers). Morphological reexamination of these and additional specimens in comparison with type materials, indicates that the initial identification of the Lengoué and Lomako River specimens as *Eugnathichthys macroterolepis* was in error, and that these fishes are in fact members of an undescribed species. Additional specimens of the undescribed species from the Luilaka River in the Salonga National Park (SNP) in central Congo and of *E. macroterolepis* and *E. eetveldii* were sequenced in order to confirm the conspecificity of the SNP specimens with the Lengoué and Lomako individuals, and also to estimate intrageneric relationships. A formal taxonomic description of the new species is provided below.

Material and methods

Species description. Fifteen morphometric measurements and 12 meristic counts were taken following Zengeya *et al.* (2011). In order to accurately count vertebral and fin-ray elements and to visualize other skeletal features, specimens were radiographed and some were cleared and stained following a modified protocol based on Taylor and Van Dyke (1985). C/S indicates cleared and stained specimens; SL denotes standard length, and HL head length. Total vertebral counts include the four modified Weberian centra but exclude the terminal, hypural-bearing centrum. Gill raker counts include only the rakers on the ceratobranchial of the first arch and exclude hypobranchial rakers and the raker in the angle of the arch. Lateral line counts exclude the small pored scales on the caudal fin distal to the point of caudal flexion. Institutional abbreviations follow Leviton *et al.* (1985).

Molecular analysis. Partial sequence fragments of glycosyltransferase (*glyt*, ncDNA) and NADH dehydrogenase subunit 2 (*ND2*, mtDNA) were amplified from total genomic DNA via polymerase chain reaction using illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare) Sequence fragments for *glyt* (Li *et al.*, 2007) were amplified using a 1:20 dilution nested PCR, with the primers Glyt_F559 (5'-GGACTGTCMAAGATGACCA CMT-3') and Glyt_R1562 (5'-CCCAAGAGGTTCTTGTTRAAGAT-3') for round 1, and Glyt_F577 (5'-ACATGG TACCAGTATGGCTTTGT-3') and Glyt_R1464 (5'-GTAAGGCATATASGTGTTCTCTCC-3') for round 2. Both rounds of nested PCR utilized the protocol 95°C initial denaturation for 1:00, followed by two elongation cycles of 20x (98°C, 0:10; 57°C, 0:30; 72°C, 0:45) and 15x (98°C, 0:10; 55°C, 0:30; 72°C, 0:45), and a final elongation of 72°C for 5:00. Sequence fragments for *ND2* were amplified from a single round of PCR, using the primers nd2_Dist_f (5'- AGCTTTTGGGCCCATACCCCA-3') and nd2_Dist_r (5'- AGGRACTAGGAGATTTTCACTCC TGCT-3') (Arroyave *et al*., in press) and the protocol 95°C initial denaturation for 1:00, followed by an elongation cycle of 35x (95°C, 1:00; 58°C, 1:00; 72°C, 2:00) and a final extension of 72°C for 10:00. Contigs of sequence chromatograms for corresponding forward and reverse reactions were assembled in Geneious v.5.1.7 (Biomatters Ltd., Aukland, NZ), checked for miscalled bases and trimmed to begin in the first codon position. Sequences were aligned by corresponding translation product using the Translation Align function in Geneious. All sequences are deposited in Genbank (Accession numbers: KF366267-KF366293).

The resulting 2-gene alignment, of length 1812 (variable sites: 234, parsimony-informative sites: 187), was partitioned by gene and codon position. Partition-optimal models of nucleotide substitution were selected using the AICc in TreeFinder (Jobb *et al.*, 2004). A maximum likelihood analysis was conducted in TreeFinder using search depth 2. After an initial topology search, 20 starting trees at nearest neighbor interchange (NNI) distances of 50 from the tree found were generated, and the analysis was re-run. Support values for the final tree were calculated using 1000 replicates of LR-ELW (Strimmer & Rambaut, 2002). A parsimony analysis was also conducted in TnT

v1.1 (Goloboff *et al.*, 2008), using the command Xmult=hits 50000 rss css fuse level 5 chklevel 10. Three equally parsimonious trees with score 302 were found. A standard parsimony bootstrap of 100 replicates was conducted on the parsimony tree.

Eugnathichthys virgatus, new species

Figures 3–5; Tables 1 & 2

Holotype: AMNH 241648, ♀, 93.00 mm SL, tissue voucher, Democratic Republic of Congo, Equateur Province, Luilaka River at confluence of small stream flowing out of Salonga National Park, 2.6713° S, 21.71452° E, Coll. R. Monsembula and R. Schelly, 11 July 2006.

Paratypes: AMNH 245002, 69.1 mm SL, Republic of Congo, Sangha Province, Lengoué River near Ouesso, Coll. V. Mamonekene, 2 September, 2007.—AMNH 246319, 51.9 mm SL, tissue voucher, Republic of Congo, Sangha Province, Lengoué River near Ouesso, Coll. V. Mamonekene, 8 September 2007.—AMNH 249790, 106.0 mm SL, tissue voucher, Democratic Republic of Congo, Equateur Province, Lomako River at Isake, 0.87944° N, 20.794722° E, Coll. Worldfish Center personnel, February 2009.—AMNH 241650, C/S, 94.8 mm SL, Democratic Republic of Congo, Equateur Province, Yenge River, Salonga National Park, 1.04605° S, 20.73328° E, Coll. R. Monsembula and R. Schelly, 28 July 2006.—MRAC B3-15-P1, 92.4 mm SL, same data as AMNH 241650.—AMNH 241649, 2 specimens, 52.2-91.4 mm SL, Democratic Republic of Congo, Equateur Province, Luilaka River at Nkema Asondji, 2.03694° S, 20.99683° E, Coll. R. Monsembula and R. Schelly, 16 July 2006.—AMNH 252256, 3 specimens, 41.0-48.0 mm SL, 3 tissue vouchers, Democratic Republic of Congo, Equateur Province, Luilaka River at Bosombangwa, Salonga National Park, 2.22435° S, 21.1851° E, Coll. R. Monsembula, 26 May 2010.—AMNH 241647, C/S, 32.1 mm SL, Democratic Republic of Congo, Equateur Province, Luilaka River at Nkomba Dumbe, 2.67111° S, 21.7211° E, Coll. R. Monsembula and R. Schelly, 09 July 2006.

Diagnosis. *Eugnathichthys virgatus*, new species, is unique among congeners in the possession of body pigmentation dominated by a broad lateral band intersected by numerous vertical bars resulting in a midlateral, checkerboard-like pigmentation patterning both in life and in preservation. Internally it is characterized by a marked reduction in dentition on the fifth ceratobranchial elements of the pharynx. Externally it differs from both congeners in the possession of a reduced total number of pectoral-fin rays (13–15 vs. 16–18). *Eugnathichthys virgatus* is further readily distinguished from *E. eetveldii* by the possession of 66–72 (vs. 96–103) pored lateral line scales from opercle to caudal flexion, 10–12 (vs. 14 or 15) scale rows between the lateral line and the dorsal-fin origin, and 8 or 9 (vs. 10) scale rows between the lateral line and pelvic-fin insertion. It differs from *E. macroterolepis*, the species that bears closest phenetic similarity, in the possession of 8 or 9 (vs. 6 or 7) scale rows between the lateral line and the pelvic-fin insertion. Diagnostic molecular characters include 4 non-synonymous, nucleotide transitions in *ND2*: T➔C (site 222); C➔A (site 530); A➔G (site 601); A➔G (site 672).

Description. A *Eugnathichthys* attaining maximum-recorded size of 106.0 mm SL (mature female, AMNH 249790), with general body shape and appearance as in Figures 3 and 4. Tables 1 and 2 summarize morphometric and meristic attributes with comparative ranges for congeners. Relatively deep-bodied, body depth 18.0–24.4 % SL (mean 20.8), greatest depth at vertical midway between pectoral and pelvic-fin insertions. Head length 29.0–31.3 % SL (mean 30.0), eyes large, bony orbit diameter 22.6–26.2 % HL (mean 24.5). Dorsal head profile straight from snout to top of head, strongly convex over nape to dorsal-fin origin. Dorsal body profile gently convex along dorsal-fin base to caudal-fin base, ventral body profile gently convex between isthmus and anal-fin base, caudal peduncle almost twice as long as deep.

Snout elongate; mouth terminal and jaws relatively massive. Contralateral premaxillae and dentaries immovably united by strongly interdigitating sutures. Upper and lower jaws both with two tooth rows. Inner row teeth small, elongate bicuspids moveably implanted in a connective tissue sheath within replacement tooth trench. Inner row tooth shafts horizontally oriented spanning tooth trench; recurved cusps vertically oriented. Outer row teeth stout, erect and closely apposed bicuspids with flattened, expanded crowns each bearing a small, blunt anterior cusp and a prominent, enlarged posterior cusp. Premaxillae and dentaries each with 15–17 outer row teeth firmly ankylosed to anterior margin of replacement trench. Premaxillary teeth overlie those on dentaries when mouth closed, resulting in a shearing bite.

TABLE 1. Morphometric data for type series of *Eugnathichthys virgatus*, new species with comparative ranges for congeners.

	Eugnathichthys virgatus, new species					*E. macroterolepis* (n =12)		*E. eetveldii (n =10)*	
	Holotype	**N**	**Mean**	**Range**	**SD**	**Mean**	**Range**	**Mean**	**Range**
Standard Length (mm)	93.0	12		32.1–106		99.0	63.4–157.0	84.3	42.3–265.0
%Standard length									
Body Depth	22.8	10	20.8	18.0–24.4	2.2	23.2	20.8–27.6	18.3	16.2–20.1
Head Length	29.0	10	30.0	29.0–31.3	0.9	29.4	27.6–30.8	27.6	25.0–30.3
Predorsal Length	48.4	10	48.9	47.6–50.5	1.0	49.4	48.3–51.6	47.7	45.6–50.0
Preanal length	76.7	10	75.0	73.0–77.1	1.5	74.3	71.5–76.3	73.9	71.4–75.5
Prepelvic length	51.8	10	52.4	50.8–55.0	1.2	52.9	51.0–55.4	52.6	51.2–55.0
Dorsal-adipose	19.1	10	19.8	18.5–20.5	1.1	20.9	19.7–22.7	17.7	17.0–18.5
Caudal peduncle length	14.3	10	14.8	13.1–16.5	1.0	15.1	13.0–16.9	16.6	14.4–19.6
Caudal peduncle depth	7.8	10	7.9	7.6–8.5	0.3	8.6	8.0–9.4	7.9	7.6–8.2
Pectoral fin length	13.7	10	14.6	13.7–16.8	0.9	15.8	14.5–16.8	14.2	13.5–15.2
%Head Length									
Eye diameter	22.6	10	24.5	22.6–26.2	1.5	22.3	20.0–25.4	26.3	24.1–28.9
Snout Length	34.1	10	32.9	30.5–34.2	1.3	32.9	30.1–35.0	31.3	28.9–34.0
Upper Jaw length	42.2	10	41.5	38.0–45.0	1.9	43.7	40.8–45.7	44.1	40.6–46.4
Interorbital width	27.0	10	24.0	21.3–25.3	2.1	24.9	21.1–28.1	25.7	23.4–29.1
Postorbital length	44.8	10	44.4	42.9–45.2	0.7	46.1	42.9–48.4	43.9	41.4–45.6

TABLE 2. Meristic data for type series of *Eugnathichthys virgatus*, new species with comparative ranges for congeners.

(*=modal count)	*E. virgatus* (n=12)	*E. macroterolepis* (n=20)	*E. eetveldii* (n=10)
Branched dorsal-fin rays	12–13 (*12)	12–13 (*12)	14–15 (*14)
Branched anal-fin rays	8–9 (*9)	9–10 (*10)	9–11 (*10)
Pectoral-fin rays (total)	13–15 (*15)	16–18 (*17)	17–18 (*17)
Gill rakers (first ceratobranchial)	9–10 (*9)	9–12 (*10)	14–17 (*16)
Lateral line scales (to flexion)	66–72 (*68)	64–68 (*66)	96–103 (*99)
Lateral line-dorsal fin scale rows	10–12 (*12)	9–12 (*10)	14–15 (*15)
Lateral line-pelvic fin scale rows	8–9 (*9)	6–7 (*7)	10–11 (*10)
Circumpeduncular scale rows	24–25 (*24)	21–24 (*24)	30–32 (31)
Total number of vertebrae	42–43 (*43)	43–45 (*44)	49
Abdominal vertebrae	26–7 (*27)	26–28 (*27)	31
Caudal vertebrae	15–16 (*16)	16–18 (*17)	18

Dorsal fin iii–iv, 12 or 13 (mode 12), anal fin iii, 8 or 9 (mode 9) with first unbranched rays diminutive, often clearly apparent only in x-rays or cleared and stained specimens. Dorsal-fin origin located well in advance of vertical through pelvic-fin insertion; first dorsal pterygiophore inserted between neural spines of vertebral centra 11 or 12. Caudal fin deeply forked, upper lobe slightly longer than lower; principal caudal-fin rays 10+9. Pectoral fin short and narrow, mean length 14.6 % HL; reduced number of 13–15 rays (count includes leading unbranched ray, Fig. 5A vs. 16–18 in congeners, Fig. 5B).

Body covered with small, regularly imbricate, distally dentate (pseudo-ctenoid) scales. Lateral line complete, in straight midlateral line from opercle to anterior margin of caudal fin, 66–72 pored scales to caudal flexion (+2–6 on caudal-fin base), 10–12 scale rows between lateral line and dorsal-fin insertion, 8 or 9 between lateral line and pelvic-fin insertion, 24 or 25 circumpeduncular scales.

Total of 9 or 10 gill rakers on ceratobranchial of first arch, rakers reduced to flattened toothplates along proximal portion of arch, becoming somewhat enlarged towards angle of arch (Fig. 6A). Toothplates on fifth ceratobranchial elements of pharynx greatly reduced, restricted to distal margin of bone and dentition limited to 2 or 3 tooth rows (Fig. 6A). Total vertebrae, 42 or 43 consisting of 27 abdominal plus 15 or 16 caudal vertebrae (holotype: 27+16). Eight supraneurals interdigitating with neural spines of last Weberian vertebra and first 7 rib-bearing vertebrae anterior to first dorsal-fin pterygiophore.

FIGURE 3. *Eugnathichthys virgatus*, new species (holotype, AMNH 241648): (A) preserved, (B) immediately post-mortem.

FIGURE 4. *Eugnathichthys virgatus*, new species (paratypes): (A) AMNH 246319, Lengoué River, (B) AMNH 249790, Lomako River.

Pigmentation and coloration. In preservation (Fig. 3A), base body coloration creamy brown, darker above midlateral line than below. Snout, upper jaw, and top of head dark brown; well marked postorbital streak passing diagonally across infraorbital 4 onto posterior margin of opercle. Lower jaw, cheek and branchiostegal membrane pale cream. One or two rows of irregular, often indistinct, oblong blotches on nape; 12–14 vertically oriented oblong bars along midlateral line, bars extended onto dorsum from mid-body to caudal peduncle. Vertical bars intersected medially by broad midlateral band resulting in characteristic checkerboard-like pigmentation

patterning. Dorsal fin pale, creamy white with three thin black stripes, adipose fin with black basal blotch and distal spotting; caudal fin with alternating black and white stripes. Remaining fins creamy white. In life (Fig. 3B), base body coloration with slight pinkish hue; iridescent silver reflections on scales. Head and body pigmentation patterning as for preserved specimens. Markings on dorsal and caudal fin as in preserved specimens but interspaces between black bands bright orange-red. Adipose fin pale orange with black spotting distally, remaining fins hyaline, with slight dusky overlay.

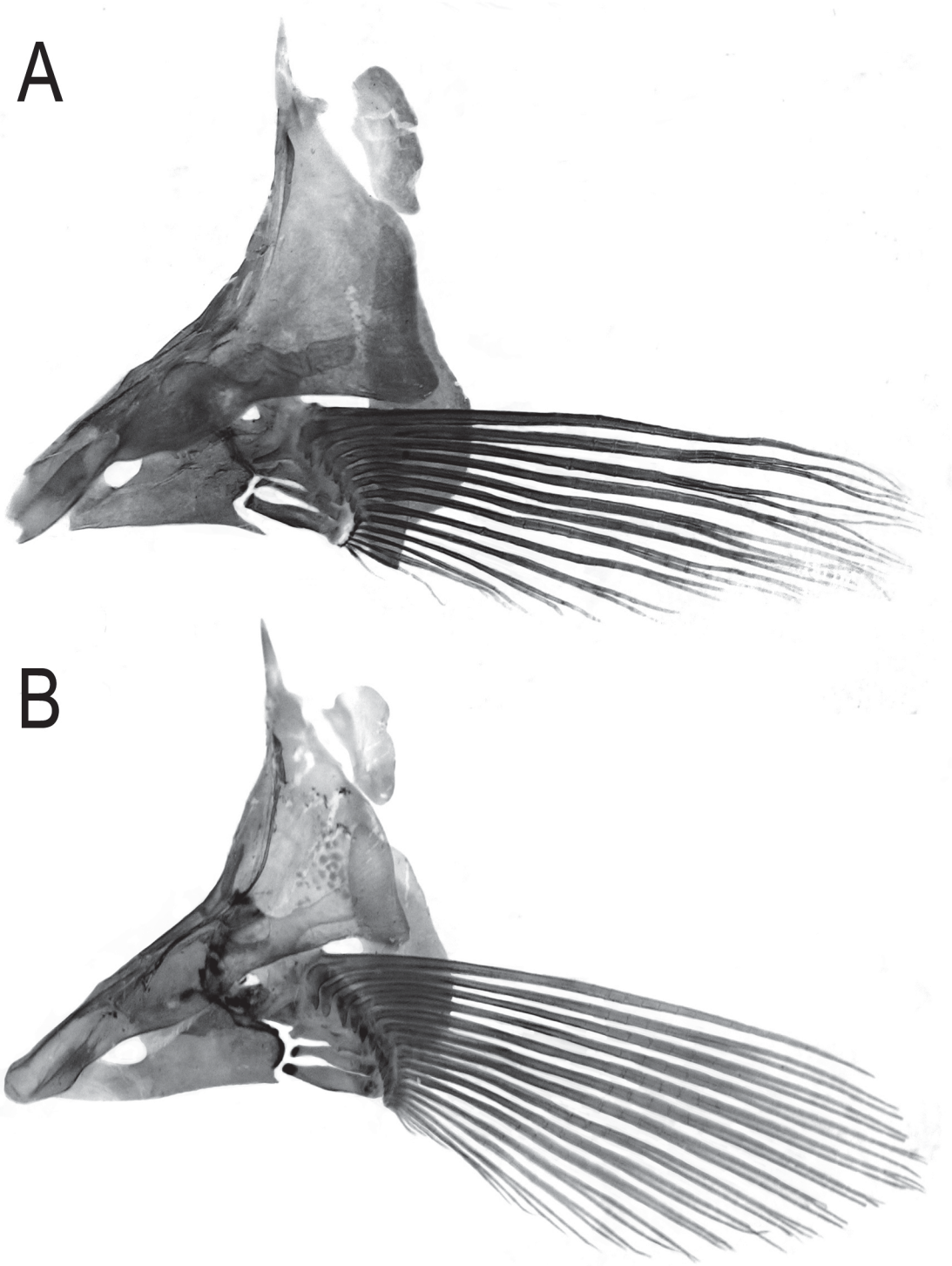

FIGURE 5. Pectoral fin and girdle (lateral view): (A) *Eugnathichthys virgatus*, AMNH 253476, 94.8 mm SL, (B) *E. macroterolepis*, AMNH 243084, 88.2 mm SL.

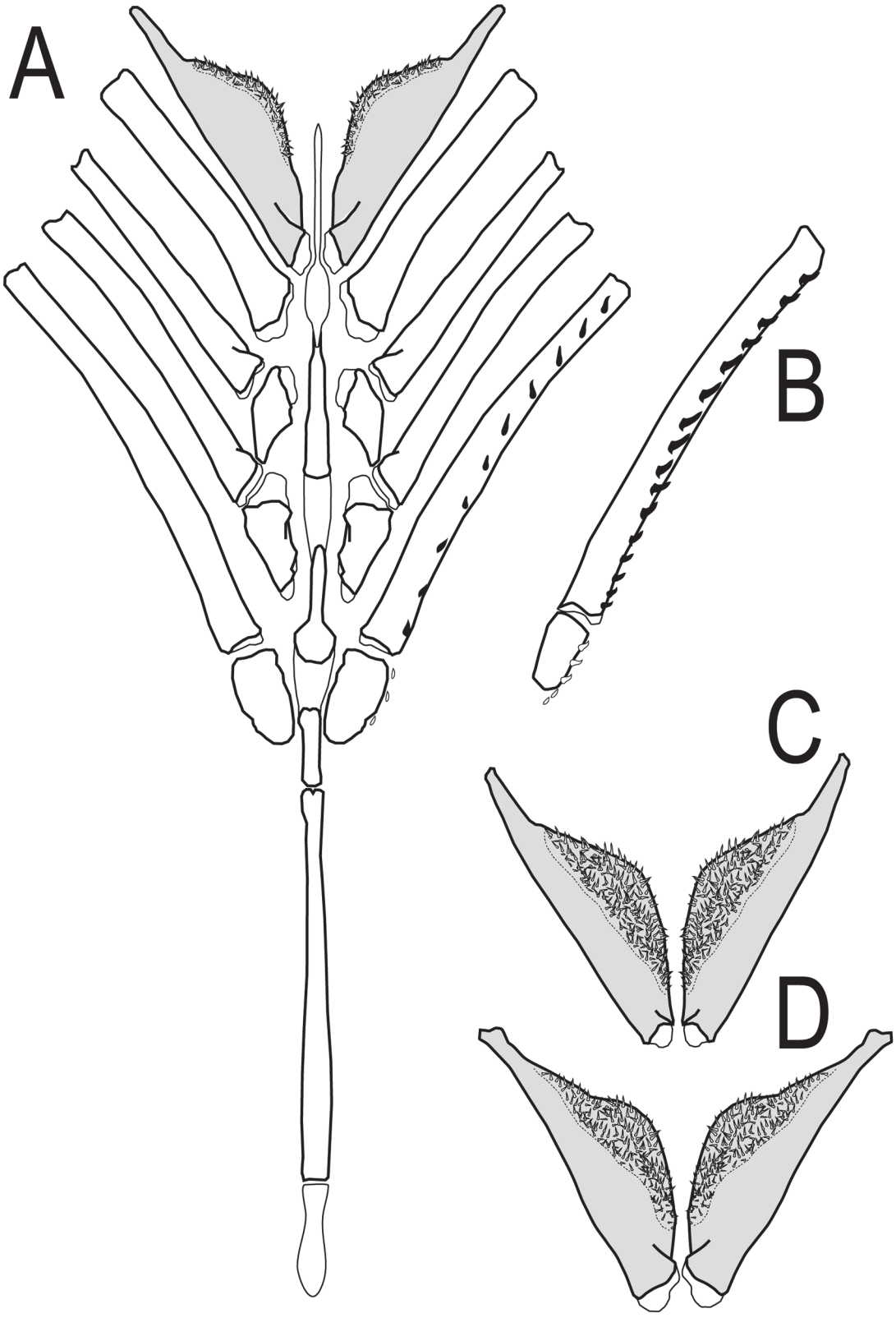

FIGURE 6. Branchial apparatus (dorsal view) fifth ceratobranchial elements shaded grey, gill rakers on first ceratobranchial, black: (A) *Eugnathichthys virgatus*, AMNH 253476, (B) *E. eetveldii*, AMNH 252993, isolated first ceratobranchial and gill rakers (black), (C) *E. macroterolepis*, AMNH 243084, isolated fifth ceratobranchials, (D) *E. eetveldii*, AMNH 252993, isolated fifth ceratobranchials.

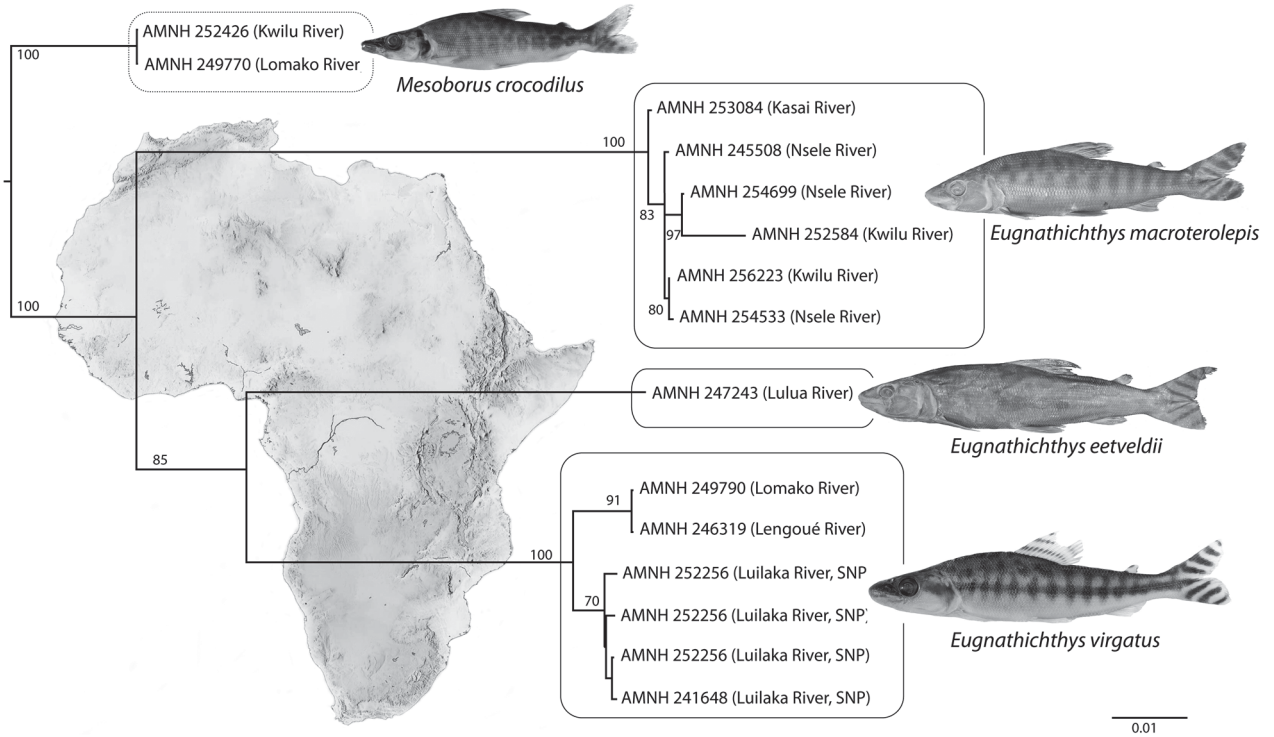

FIGURE 7. *Eugnathichthys* species relationships. Indices at nodes indicate LR-ELW resampling support >50. Species exemplars inset: *Mesoborus crocodilus* (AMNH 251761), *Eugnathichthys macroterolepis* (holotype, BMNH 1899.6.28:16), *E. eetveldii* (syntype, MRAC 148), and *E. virgatus* (holotype, AMNH 241648).

Geographical variation. Preceding description based on holotype and paratypes from SNP. Specimens from Lengoué River (Fig. 4A) and Lomako River (Fig. 4B) are darker than Salonga conspecifics, interspaces on dorsal fin greatly reduced, and in Lengoué specimens ventrum, pectoral, pelvic and anal fins are sooty black. Additionally, caudal fin pigmentation of Lomako specimen is strongly reticulate not striped. Despite these differences, all populations exhibit body pigmentation dominated by a broad lateral band intersected by vertical bars, here considered diagnostic of the species.

Distribution. Currently known only from three regions in western and central Congo basin; Lengoué River near Ouesso, Republic of Congo (Fig. 1, area 1), Lomako River at Isake, Democratic Republic of Congo (Fig. 1, area 2), and localities along the Luilaka and Yenge Rivers in the SNP, Democratic Republic of Congo (Fig. 1, area 3). While most of these areas are poorly known and their ichthyofaunas have yet to be fully documented (Stiassny *et al.*, 2011; Monsembula Iyaba & Stiassny, 2013), from collections housed at the AMNH it is evident that *E. macroterolepis* also occurs in the Lengoué. Yet despite relatively intensive recent collecting efforts in the SNP *E. virgatus* is the only *Eugnathichthys* currently recorded from that region. Given the disjunct locations from which the species has been recorded it is probable that *E. virgatus* is more widespread than currently known. We note, for example that Roberts (1990: Fig. 3) provides a photograph of a 70 mm individual identified as *E. macroterolepis* from the Mossapoula River (Ubangi drainage) (Fig. 1, region depicted with "??"). We have been unable to locate this specimen in a museum collection, but from the illustration it clearly exhibits the characteristic pigmentation patterning of *E. virgatus*—a broad midlateral band intersected by numerous vertical bars—suggesting the range of *E. virgatus* extends into the Ubangi basin.

Feeding. While field observations were not made, gut morphology and contents suggest that the species feeds exclusively on fish fins. The stomach is a large, simple sac of length 20–25 % SL, from which a short gut issues basally and coils twice before exiting the body cavity. The unraveled combined length of stomach and intestine is about 1.25 times SL. All specimens regardless of size or geographical location, contained pieces of fish fin packaged together and often filling the stomach; no other food items were found. Roberts (1990) observed that young *E. eetveldii* feed on aquatic insect larvae, and that the smallest specimen with fins observed in the stomach was an individual of 96 mm SL. In this study, a juvenile *E. eetveldii* (AMNH 247243, 41 mm SL) was found to have a mixture of fish fins and aquatic insect larvae in its stomach, indicating that *E. eetveldii* may undergo an ontogenetic shift in trophic strategy. This does not appear to be the case for *E. virgatus* as all juveniles, even the

smallest examined (32.1 mm SL), apparently feed exclusively on fins. Both caudal and unpaired fin fragments were recovered but unfortunately it was not possible to discern the prey species. We note that larger individuals of *E. virgatus* are missing large portions of their dorsal fins (see Figs 3 and 4), but whether this is a result of intraspecific ectoparasitism, or from attack by sympatric fin-biting distichodontids is unclear.

Ecology and habitat. All specimens of *E. virgatus* were collected exclusively in heavily shaded, highly humic forest rivers.

Etymology. *Virgatus*, from the Latin, in reference to the conspicuous midlateral band or streak forming the characteristic pigmentation patterning of the species in life and in preservation.

Molecular phylogenetic analysis

In order to confirm that SNP specimens were conspecific with the Lengoué and Lomako individuals, and to investigate species relationships within the genus, a small-scale phylogenetic analysis was undertaken. Based on the phylogenetic hypothesis of Vari (1979) and our own preliminary analyses, the genus *Mesoborus* was selected as an appropriate outgroup for rooting the *Eugnathichthys* tree. Results of a maximum likelihood analysis in TreeFinder (Jobb *et al.*, 2004), based on a 2-gene alignment partitioned by gene and codon position, are presented in Figure 7, and an identical topology was retrieved in parsimony reconstruction in TnT (Goloboff *et al.*, 2008). The SNP samples cluster together with the Lengoué and Lomako individuals forming two somewhat geographically differentiated subclades within a strongly supported monophyletic *E. virgatus*. *Eugnathichthys virgatus* is further resolved as sister to *E. eetveldii*, although support is somewhat lower than that supporting monophyly of *E. macroterolepis* and *E. virgatus*.

Comparative materials

Eugnathichthys eetveldi: MRAC 148, Syntype, Upoto.—MRAC 121, Syntype, Leopoldville.—MRAC 26. Syntype, Boma.—ZMB 19043, Holotype of *Eugnathichthys intermedius*, Aruwimi River at Basoko.—AMNH 5804, 1 specimen, Stanleyville, junction of Lualaba with Congo River.—AMNH 5899, 1 specimen, Stanleyville, junction of Lualaba with Congo River.—AMNH 227473, 1 specimen, Bobongo creek into Sangha River.—AMNH 227513, 1 specimen, Middle of Sangha River at Bayonga.—AMNH 243649, 1 specimen, Katende, Lulua River.—AMNH 252993, 1 specimen, C/S, Lulua River at Nsanga Nyembo.—AMNH 253161, 1 specimen, Lulua River at Nsanga Nyembo.—AMNH 247243, 1 specimen, Lulua River upstream of confluence with Kasai River.

Eugnathichthys macroterolepis: BMNH 1899.6.28:16, Holotype, Chiloango, Angola.—AMNH 5810, 1 specimen, Stanleyville, junction of Lualaba with Congo River.—AMNH 6148, 1 specimen, Avakubi, Ituri River.—AMNH 6069, 1 specimen, Rungu, Bomokandi River.—AMNH 6347, 1 specimen, Poko, Bomokandi River.—AMNH 5999, 1 specimen, Faradji, Dungu River.—AMNH 6332, 1 specimen, Malela, mouth of Congo River.—AMNH 12421, 1 specimen, Lulua River, Luluabourg.—AMNH 238341, 2 specimens, Upstream of Boma, Congo River.—AMNH 245003, 2 specimens, Lengoué River, Upstream of Ouesso.—AMNH 245004, 1 specimen, Sangha River at Point 9.—AMNH 245588, 1 specimen, Nsele River at market.—AMNH 252994, 1 specimen, Lulua River at Nsanga Nyembo.—AMNH 252805, 1 specimen, Lulua River at Mukundulu.—AMNH 253084, 1 specimen, C/S, Kasai River at Tshikapa.—AMNH 253624, 3 specimens, 1C/S, Kwilu River at Kwilu beach.—AMNH 252378, 1 specimen, Nsele River at Koke ya Mbila.—AMNH 252584, 1 specimen, Kwilu River at Kikwit.

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